# PCT

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| (51) International Patent Classification 6 : A61K 48/00, C07H 21/00, C12N 15/00, C12P 21/04, 21/06, 21/08 | A1 | (11) International Publication Number: WO 98/44955 |
|---|---|---|
| | | (43) International Publication Date: 15 October 1998 (15.10.98) |

(21) International Application Number: PCT/US98/06900

(22) International Filing Date: 9 April 1998 (09.04.98)

(30) Priority Data:
60/041,850        9 April 1997 (09.04.97)        US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
*With international search report.*
*Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

(54) Title: RECOMBINANT ANTIBODIES SPECIFIC FOR BETA-AMYLOID ENDS, DNA ENCODING AND METHODS OF USE THEREOF

(57) Abstract

DNA encoding a recombinant antibody molecule end-specific for an amyloid-$\beta$ peptide, pharmaceutical compositions thereof, and a method for preventing or inhibiting progression of Alzheimer's Disease by introducing such a DNA molecule into brain cells to express the recombinant antibody molecule and prevent the accumulation of amyloid-$\beta$ peptides in the cerebrospinal fluid.

# RECOMBINANT ANTIBODIES SPECIFIC FOR BETA-AMYLOID ENDS, DNA ENCODING AND METHODS OF USE THEREOF

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## CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority under 35 USC 119(e) from U.S. provisional application no. 60/041,850,
10 filed April 9, 1997, the entire contents of which are hereby incorporated by reference.

## BACKGROUND OF THE INVENTION
### Field of the Invention
15 The present invention relates to a method for preventing or inhibiting progression of Alzheimer's Disease through gene delivery to cells of the central nervous system. The present invention also relates to a recombinant DNA molecule containing a gene encoding a recombinant antibody
20 molecule end-specific for an amyloid-$\beta$ peptide operably-linked to a promoter capable of expressing a recombinant antibody in cells of the central nervous system, and pharmaceutical compositions thereof.

25 ### Description of the Background Art
A major histopathological hallmark of Alzheimer's Disease (AD) is the presence of amyloid deposits within neuritic and diffuse plaques in the parenchyma of the amygdala, hippocampus and neocortex (Glenner and Wong, 1984;
30 Masters et al., 1985; Sisodia and Price, 1995). Amyloid is a generic term that describes fibrillar aggregates that have a common $\beta$-pleated structure. These aggregates exhibit birefringent properties in the presence of Congo red and polarized light (Glenner and Wong, 1984). The diffuse plaque
35 is thought to be relatively benign in contrast to the neuritic plaque which appears to be strongly correlated with reactive and degenerative processes (Dickson et al., 1988; Tagliavini et al., 1988; Yamaguchi et al., 1989; Yamaguchi et al., 1992). The principal component of neuritic plaques is a

42 amino acid residue amyloid-$\beta$ (A$\beta$) protein (Miller et al.,
1993; Roher et al., 1993) that is derived from the much
larger $\beta$-amyloid precursor protein, $\beta$APP (or APP) (Kang et
al., 1987). A$\beta$ 1-42 is produced less abundantly than the 1-
40 A$\beta$ peptide (Haass et al., 1992; Seubert et al., 1992), but
the preferential deposition of A$\beta$1-42 results from the fact
that this COOH-extended form is more insoluble than 1-40 A$\beta$
and is more prone to aggregate and form anti-parallel $\beta$-
pleated sheets (Joachim et al., 1989; Halverson et al., 1990;
Barrow et al., 1992; Burdick et al., 1992; Fabian et al.,
1994). A$\beta$1-42 can seed the aggregation of A$\beta$ 1-40 (Jarrett
and Lansbury 1993).

The APP gene was sequenced and found to be encoded
on chromosome 21 (Kang et al., 1987). Expression of the APP
gene generates several A$\beta$-containing isoforms of 695, 751 and
770 amino acids, with the latter two $\beta$APP containing a domain
that shares structural and functional homologies with Kunitz
serine protease inhibitors (Kang et al., 1987; Kitaguchi et
al., 1988; Ponte et al., 1988; Tanzi et al., 1988; Konig et
al., 1992). The functions of $\beta$APP in the nervous system
remain to be defined, although there is increasing evidence
that $\beta$APP has a role in mediating adhesion and growth of
neurons (Schubert et al., 1989; Saitoh et al., 1994; Saitoh
and Roch, 1995) and possibly in a G protein-linked signal
transduction pathway (Nishimoto et al., 1993). In cultured
cells, $\beta$APPs mature through the constitutive secretory
pathway (Weidemann et al., 1989; Haass et al., 1992; Sisodia
1992) and some cell-surface-bound $\beta$APPs are cleaved within
the A$\beta$ domain by an enzyme, designated $\alpha$-secretase, (Esch et
al., 1990), an event that precludes A$\beta$ amyloidogenesis.
Several studies have delineated two additional pathways of
$\beta$APP processing that are both amyloidogenic: first an
endosomal/lysosomal pathway generates a complex set of $\beta$APP-
related membrane-bound fragments, some of which contain the
entire A$\beta$ sequence (Haass et al., 1992; Golde et al., 1992);
and second, by mechanisms that are not fully understood, A$\beta$
1-40 is secreted into the conditioned medium and is present
in cerebrospinal fluid *in vivo* (Haass et al., 1992; Seubert

et al., 1992; Shoji et al., 1992; Busciglio et al., 1993).
Lysosomal degradation is no longer thought to contribute
significantly to the production of A$\beta$ (Sisodia and Price,
1995). The proteolytic enzymes responsible for the cleavages
5  at the $NH_2$ and COOH termini of A$\beta$ termed $\beta$ and $\gamma$,
respectively, have not been identified. Until recently, it
was generally believed that A$\beta$ is generated by aberrant
metabolism of the precursor. The presence, however, of A$\beta$ in
conditioned medium of a wide variety of cells in culture and
10  in human cerebrospinal fluid indicate that A$\beta$ is produced as
a normal function of cells.

If amyloid deposition is a rate-limiting factor to
produce AD, then all factors linked to the disease will
either promote amyloid deposition or enhance the pathology
15  that is provoked by amyloid. The likelihood of amyloid
deposition is enhanced by trisonomy 21 (Down's syndrome)
(Neve et al., 1988; Rumble et al., 1989), where there is an
extra copy of the APP gene, by increased expression of APP,
and by familial Alzheimer's Disease (FAD)-linked mutations
20  (Van Broeckhoven et al., 1987; Chartier-Harlin et al., 1991;
Goate et al., 1989; Goate et al., 1991; Murrell et al., 1991;
Pericak-Vance et al., 1991; Schellenberg et al., 1992; Tanzi
et al., 1992; Hendricks et al., 1992; Mullan et al., 1992).
Some of these mutations are correlated with an increased
25  total production of A$\beta$ (Cai et al., 1993; Citron et al.,
1992) or specific overproduction of the more fibrillogenic
peptides (Wisniewski et al., 1991; Clements et al., 1993;
Susuki et al., 1994) or increased expression of factors that
induce fibril formation (Ma et al., 1994; Wisniewski et al.,
30  1994). Collectively, these findings strongly favor the
hypothesis that amyloid deposition is a critical element in
the development of AD (Hardy 1992), but of course they do not
preclude the possibility that other age-related changes
associated with the disease, such as paired helical
35  filaments, may develop in parallel rather than as a result of
amyloid deposition and contribute to dementia independently.

The main focus of researchers and the principal aim
of those associated with drug development for AD is to reduce

the amount of Aβ deposits in the central nervous system
(CNS). These activities fall into two general areas:
factors affecting the production of Aβ, and factors affecting
the formation of insoluble Aβ fibrils. A third therapeutic
5   goal is to reduce inflammatory responses evoked by Aβ
neurotoxicity.

With regards to the first, a major effort is
underway to obtain a detailed understanding of how newly
synthesized βAPP is processed for insertion into the plasma
10  membrane and to identify the putative amyloidogenic
secretases that have been assigned on the basis of sites for
cleavage in the mature protein. From a pharmacological
perspective, the most direct way of reducing the production
of Aβ is through direct inhibition of either β or γ
15  secretase. No specific inhibitors are currently available
although a number of compounds have been shown to indirectly
inhibit the activities. Bafilomycin, for example, inhibits
Aβ production with an $EC_{50}$ of about 50 nM (Knops et al., 1995;
Haass et al., 1995), most likely through its action as an
20  inhibitor of vacuolar $H^+$ATPase co-localized in vesicles with
the Aβ secretase. Another compound, MDL28170, used at high
concentrations appears to block the activity of γ secretase
Higaki et al., 1995). It is generally hoped that the
identification of the β or γ secretases might lead to the
25  synthesis of specific protease inhibitors to block the
formation of amyloidogenic peptides. It is not known,
however, whether these enzymes are specific for βAPP or
whether they perhaps have other important secretory
functions.                    Similarly, problems of target and
30  targeting specificity will be encountered through any attempt
to interfere with signal transduction pathways that may
determine whether processing of βAPP is directed through the
amyloidogenic or non-amyloidogenic pathways. Moreover, these
signal transduction mechanisms still need to be identified.
35  In conclusion, present understanding of the complex and
varied underlying molecular mechanisms leading to
overproduction of Aβ offers little hope for selective
targeting by pharmacological agents.

Given that neurotoxicity appears to be associated with β-pleated aggregates of Aβ, one therapeutic approach is to inhibit or retard Aβ aggregation. The advantage of this approach is that the intracellular mechanisms triggering the overproduction of Aβ or the effects induced intracellularly by Aβ need not be well understood. Various agents that bind to Aβ are capable of inhibiting Aβ neurotoxicity in vitro, for example, the Aβ-binding dye, Congo Red, completely inhibits Aβ-induced toxicity in cultured neurons (Yankner et al., 1995). Similarly, the antibiotic rifampacin also prevents Aβ aggregation and subsequent neurotoxicity (Tomiyama et al., 1994). Other compounds are under development as inhibitors of this process either by binding Aβ directly, e.g., hexadecyl-N-methylpiperidinium (HMP) bromide (Wood et al., 1996), or by preventing the interaction of Aβ with other molecules that contribute to the formation of Aβ deposition. An example of such a molecule is heparan sulfate or the heparan sulfate proteoglycan, perlecan, which has been identified in all amyloids and is implicated in the earliest stages of inflammation associated amyloid induction.

Heparan sulfate interacts with the Aβ peptide and imparts characteristic secondary and tertiary amyloid structural features. Recently, small molecule anionic sulfates have been shown to interfere with this reaction to prevent or arrest amyloidogenesis (Kisilevsky, 1995), although it is not evident whether these compounds will enter the CNS. A peptide based on the sequence of the perlecan-binding domain appears to inhibit the interaction between Aβ and perlecan, and the ability of Aβ-derived peptides to inhibit self-polymerization is being explored as a potential lead to developing therapeutic treatments for AD. The effectiveness of these compounds in vitro, however, is likely to be modest for a number of reasons, most notably the need for chronic penetration of the blood brain barrier.

As another means of inhibiting or retarding Aβ aggregation, WO 96/25435 discloses the potential for using a monoclonal antibody, which is end-specific for the free C-

terminus of the Aβ 1-42 peptide, but not for the Aβ 1-43
peptide, in preventing the aggregation of Aβ 1-42. While the
administration of such an Aβ end-specific monoclonal antibody
is further disclosed to interact with the free C-terminal

5   residue of Aβ 1-42, thereby interfering with and disrupting
aggregation that may be pathogenic in AD, there is no
specific disclosure on how these Aβ C-terminal-specific
monoclonal antibodies would be used therapeutically.
Although direct or indirect manipulation of Aβ peptide

10  aggregation appears to be an attractive therapeutic strategy,
a possible disadvantage of this general approach may be that
pharmacological compounds of this class will need to be
administered over a long period of time, and may accumulate
and become highly toxic in the brain tissue.

15          An alternative to a peptide-based approach is to
elucidate the cellular mechanism of Aβ neurotoxicity and
develop therapeutics aimed at those cellular targets. The
focus has been on controlling calcium dysfunction of free
radical mediated neuronal damage. It has been postulated

20  that Aβ binds to RAGE (the receptor for advanced glycation
end-products) on the cell surface, thereby triggering
reactions that could generate cytotoxic oxidizing stimuli
(Yan et al., 1996). Blocking access of Aβ to the cell
surface binding site(s) might retard progression of neuronal

25  damage in AD. To date there are no specific pharmacological
agents for blocking Aβ-induced neurotoxicity.

            In addition to therapeutic approaches through the
direct administration of pharmacologically active agents, WO
89/01975 discloses a method of transplanting glial cells

30  (actively secreting cells derived from within the brain),
which have been transformed to express and secrete
recombinant polymeric anti-acetylcholinesterase antibodies of
the IgM class. It is predicted in the disclosure of WO
89/01975 that the antibody secreted by the transformed cells

35  transplanted into the brain of a person suffering from
Alzheimer's Disease may then alleviate or abolish the
symptoms of the disease. This is a gene therapeutic approach
arising from the observation that cells of the central

nervous system are very efficient in the secretion of
antibodies (Cattaneo and Neuberger, 1987). Piccioli et al.,
1991 and 1995, later demonstrated the ectopic neuronal
expression of recombinant antibodies from the promoter of the
5    neuronal *vgf* gene in a tissue-specific and developmentally
regulated manner. Thus, non-lymphoid cells, and in
particular, neuronal cells were found to be capable of
secreting functional immunoglobulins.

       Citation of any document herein is not intended as
10   an admission that such document is pertinent prior art, or
considered material to the patentability of any claim of the
present application. Any statement as to content or a date
of any document is based on the information available to
applicant at the time of filing and does not constitute an
15   admission as to the correctness of such a statement.

## SUMMARY OF THE INVENTION

       The present invention relates to a novel method for
preventing the onset of Alzheimer's Disease or for inhibiting
20   progression of Alzheimer's Disease through the stable
expression in the brain of recombinant antibodies end-
specific for amyloid-$\beta$ peptides. These ectopically expressed
recombinant antibody molecules, which are end-specific for
the N-terminus or C-terminus of amyloid-$\beta$ peptides, prevent
25   the accumulation of amyloid-$\beta$ peptides in the extracellular
space, interstitial fluid and cerebrospinal fluid and the
aggregation of such peptides into amyloid deposits in the
brain. Given the many possible mechanisms that might
contribute to the production of amyloid-$\beta$, coupled with the
30   tremendous diversity of interactions of A$\beta$ with the cell
surface and extracellular A$\beta$-binding molecules capable of
bringing about chronic neurotoxicity, the present method is
directed to preventing the accumulation of A$\beta$ peptides in the
extracellular milieu of affected neurons as the focal point
35   of this heterogeneous pathological cascade. The present
invention also avoids the problems associated with the
repeated administration of pharmacological agents that
requires chronic penetration of the blood brain barrier.

It is therefore an object of the invention to overcome the deficiencies in the prior art by providing a novel method for preventing or inhibiting the progression of Alzheimer's Disease.

5      Another object of the invention is to provide a method whereby cells of the nervous system are conferred with the ability to ectopically express recombinant antibody molecules in the brain, which molecules are end-specific for the N-terminus or C-terminus of amyloid-β peptides, to

10    prevent the accumulation of amyloid-β peptides in the extracellular space, interstitial fluid and cerebrospinal fluid and the aggregation of such peptides into amyloid deposits in the brain.

A further object of the invention is to provide a

15    method for preventing or inhibiting the progression of Alzheimer's Disease by also inhibiting the interaction of amyloid-β peptides mediating amyloid-β induced neurotoxicity and inhibiting the amyloid-β induced complement activation and cytokine release involved in the inflammatory process

20    associated with Alzheimer's Disease.

Still another object of the invention is to provide a recombinant DNA molecule, containing a gene encoding a recombinant antibody molecule end-specific for the N-terminus or the C-terminus of an amyloid-β peptide and operably-linked

25    to a promoter which is expressed in the central nervous system.

Yet another object of the invention is to provide a vector for introducing the recombinant DNA molecule into cells of the central nervous system.

30    Still yet another object of the invention is to provide a pharmaceutical composition for preventing or inhibiting the progression of Alzheimer's Disease.


## BRIEF DESCRIPTION OF THE DRAWINGS

35    Figure 1 shows a schematic representation of the β-amyloid precursor protein (βAPP) and the products of α, β, and γ-secretase cleavage. The general locations of various domains are indicated along with the cleavage sites (α, β, γ)

for secretases.  Figure 1 also schematically shows that the
stable expression and secretion of ectopic Aβ-end-specific
antibodies in the CNS inhibits (1) the accumulation of Aβ
peptides and (2) the neurotoxic consequences of amyloid
5      deposition without affecting the biological functions of the
soluble β-amyloid precursor protein.

Figure 2 shows the amino acid sequence (SEQ ID
NO:1) of the region in βAPP from which β-amyloid peptides
(Aβ) are derived.  The arrows indicate the α-, β- or γ-
10     secretase cleavage sites, and the amino acid residues
corresponding to the synthetic peptides to be used as
immunogens are indicated underneath the sequence by line
segments.

Figures 3A-3D schematically show the structure of a
15     whole antibody (Fig. 3A) with the variable domain of heavy
($V_H$) and light ($V_L$) chains and the constant domain(s) of light
($C_L$) and heavy ($C_H1$, $C_H2$, $C_H3$) chains, a Fab fragment (Fig.
3B), a Fv fragment (Fig. 3C), and a single chain Fv fragment
(scFv) (Fig. 3D).  The Fab fragment shown in Fig. 3B consists
20     of a variable domain of heavy $V_H$ and light $V_L$ chain and the
first constant domain ($C_H1$ and $C_L$) joined by a disulfide
bridge.  The Fv fragment shown in Fig. 3C represents the
antigen binding portion of an antibody formed by a non-
covalently linked variable region complex ($V_H$-$V_L$), whereas the
25     single chain Fv shown in Fig. 3D joins the variable heavy $V_H$
with the variable light $V_L$ chain via a peptide linker.

Figure 4 schematically shows the construction of a
scFv antibody by cloning the variable region of an end-
specific anti-Aβ monoclonal antibody using the PCR
30     amplification technique with primers A, B, C and D, and then
joining together the variable heavy $V_L$ chain and the variable
light $V_L$ chain with an interchain peptide linker (ICL).  The
shaded area represents hypervariable regions of the antigen
binding site and LP designates the leader peptide of the
35     heavy and light chains.

Figure 5 shows a schematic representation of the
AAV ScFvαAβ vector with the inverted terminal repeats (ITR),

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human βAPP promoter (HuβAPPP), SV40 polyadenylation signal
(SV40pA) indicated.   The plasmid backbone is pSSV9.

## DETAILED DESCRIPTION OF THE INVENTION

5          The novel DNA molecules of the present invention
contain a gene encoding a recombinant antibody molecule end-
specific for the N-terminus or the C-terminus of an Aβ
peptide.   Such a recombinant antibody molecule discriminates
between an Aβ peptide and the β-amyloid protein precursor
10    from which it is proteolytically derived, and is also
referred to throughout the present specification as an
"antisenilin".  By "antisenilin" is meant a molecule which
binds specifically to a terminus/end of an Aβ peptide to slow
down or prevent the accumulation of amyloid-β peptides in the
15    extracellular space, interstitial fluid and cerebrospinal
fluid and the aggregation into senile amyloid deposits or
plaques and to block the interaction of Aβ peptides with
other molecules that contribute to the neurotoxocity of Aβ.
           The method for preventing or inhibiting the
20    progression of Alzheimer's Disease in accordance with the
present invention, involves delivering the gene encoding the
antisenilin molecule into brain cells where antisenilins are
then stably expressed and secreted into the extracellular
space, interstitial fluid and cerebrospinal fluid.   The
25    secretion of antisenilins into the extracellular space,
interstitial fluid and cerebrospinal fluid, where soluble Aβ
peptides are present, promotes the formation of soluble
antisenilin-Aβ complexes.   These soluble antisenilin-Aβ
complexes are cleared from the central nervous system by
30    drainage of the extracellular space, interstitial fluid and
cerebrospinal fluid into the general blood circulation
through the arachnoid villi of the superior sagittal sinus.
In this manner, soluble Aβ peptides are prevented from
accumulating in the extracellular space, interstitial fluid
35    and cerebrospinal fluid to form amyloid deposits and/or to
induce neurotoxicity (Fig. 1).   Furthermore, clearance of
soluble amyloid-β peptides in accordance with the present
invention is expected to reduce the inflammatory process

observed in Alzheimer's Disease by inhibiting, for example, amyloid-β-induced complement activation and cytokine release, and block also the interaction of Aβ with cell surface receptors such as the RAGE receptor.

5          The composition of the present invention includes a recombinant DNA molecule containing an antisenilin gene in association with a means for gene delivery where this composition may be for use as a medicament for preventing or inhibiting the progression of Alzheimer's Disease.

10         As shown in Fig. 1 (see Schehr, 1994), and discussed in the Background Art section, the β-amyloid protein precursor (βAPP) is believed also to serve as a precursor for a proteolytic product, soluble β-amyloid protein precursor (sβAPP), thought to have growth promoting

15   and neuroprotective functions. It will be readily appreciated by those of skill in the art that the stable expression of antisenilins in the central nervous system will not interfere with the normal biological functions of βAPP that are not associated with the formation of Aβ peptides.

20   In the novel recombinant DNA molecules of the present invention, the gene encoding an antisenilin molecule contains at least the nucleotide sequences which encode the antigen-binding domain of an end-specific monoclonal antibody molecule. Thus, the antisenilin molecule, which is a

25   recombinant antibody molecule containing the antigen-binding portion of a monoclonal antibody, is intended to encompass a chimeric or humanized immunoglobulin molecule of any isotype, as well as a single-chain antibody.

Chimeric antibodies are understood to be molecules,
30   different portions of which are derived from different animal species, such as those having a variable region derived from a mouse monoclonal antibody and a human immunoglobulin constant region. Chimeric antibodies and methods for their production are well known in the art. For example, the DNA

35   encoding the variable region of the antibody can be inserted into or joined with DNA encoding other antibodies to produce chimeric antibodies (U.S. patent 4,816,567; Orlandi et al., 1989).

Single-chain antibodies as antisenilins can also be
produced according to the present invention.  These single
chain antibodies can be single chain composite polypeptides
having end-specific Aβ peptide binding capability and

5    comprising a pair of amino acid sequences homologous or
analogous to the variable regions of an immunoglobulin light
and heavy chain (linked $V_H$-$V_L$ or single chain Fv).  Both $V_H$
and $V_L$ may copy natural monoclonal antibody sequences, or one
or both of the chains may comprise a CDR-FR construct of the

10   type described in U.S. Patent 5,091,513.  The separate
polypeptides analogous to the variable regions of the light
and heavy chains are held together by a peptide linker.
Methods of production of such single chain antibodies, e.g.,
single chain Fv (scFv), particularly where the DNA encoding

15   the polypeptide structures of the $V_H$ and $V_L$ chains are
characterized or can be readily ascertained by sequence
analysis, may be accomplished in accordance with the methods
described, for example, in U.S. Patent 4,946,778, U.S. Patent
5,091,513, U.S. Patent 5,096,815, Biocca et al., 1993, Duan

20   et al., 1994, Mhashilkar et al., 1995, Marasco et al., 1993,
and Richardson et al., 1995.  Figures 3A-3D (from Biocca et
al., 1995) schematically show an intact antibody (Fig. 3A), a
Fab fragment (Fig. 3B), a Fv fragment consisting of a non-
covalently linked variable region complex ($V_H$-$V_L$ (Fig. 3C),

25   and a single chain Fv antibody (Fig. 3D).

In constructing the recombinant gene encoding the
antisenilin molecule, a hybridoma producing a monoclonal
antibody end-specific for the N-terminus or C-terminus of an
amyloid-β peptide is first obtained, where an end-specific

30   antibody is defined as an antibody which uniquely recognizes
the free N-terminus or the free C-terminus of a peptide and
which can further discriminate between the peptide and the
precursor from which it is proteolytically derived.  The
design of immunogenic peptides for use in immunization and

35   the generation of monoclonal antibody producing hybridomas is
based on similar peptides that have been previously used by
several laboratories to generate monoclonal antibodies that
uniquely recognize the free amino or carboxy-terminal of Aβ

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(Harrington et al., 1993; Iwatsubo et al., 1994; Konig et
al., 1996; Murphy et al., 1994; Gravina et al., 1995). While
peptides of longer lengths have in some instances been used
successfully to generate Aβ end-specific antibodies, Saido

5    and co-workers (1993; 1994) established that there is a
length of five amino acids for any given peptide which
ensures that the specific free amino group at the N-terminus
constitutes an essential part of the epitope recognized by
the new antibody.  Thus, a monoclonal antibody generated

10   against an immunogenic peptide is evaluated for the
selectivity of the antibody in its recognition of a free N-
or C-terminus of an Aβ peptide.  A competitive inhibition
assay, using Enzyme-Linked Immunosorbant Assay (ELISA) or
immunoprecipitation with peptides corresponding to different

15   regions of Aβ and the region immediately preceding the β-
secretase cleavage site in the extracellular domain of βAPP,
can determine the selectivity of the monoclonal antibody.
When clearance of the amyloid peptides involved in the
pathogenesis of Alzheimer's Disease, i.e., Aβ1-40

20   (corresponding to residues 5-44 of SEQ ID NO:1), Aβ1-42
(corresponding to residues 5-46 of SEQ ID NO:1), and Aβ1-43
(corresponding to residues 5-47 of SEQ ID NO:1), is the major
goal, then the monoclonal antibody is preferably end-specific
for the N-terminus that is common to these Aβ peptides.  In

25   other cases, however, such as when used to treat a patient
following the onset of Alzheimer's Disease, it may be
preferable to select an antibody that will also interfere
with the ability of Aβ peptides to seed aggregation or to
interact with other molecules that either contribute to the

30   seeding of Aβ deposition or mediate Aβ-induced cytotoxic
effects.  Immunogenic peptides of varying lengths, which
incorporate either the free N-terminus or free C-terminus,
are synthesized to allow for generating end-specific anti-Aβ
antibodies and the recombinant DNA encoding for a recombinant

35   Aβ end-specific antibody (antisenilin) used in a
pharmaceutical composition for this type of selective
application in preventing or inhibiting the progression of
Alzheimer's Disease.

Those of skill in the art will appreciate that a cysteine residue can be added to the end of the above immunogenic peptides opposite from the end corresponding to the free N-terminus or the free C-terminus of Aβ peptides to facilitate coupling to a carrier protein. Keyhole limpet hemocyanin (KLH), ovalbumin and bovine serum albumin (BSA) are non-limiting examples of proteins that can be used as carriers for immunogens. The presence of an N-terminal or C-terminal cysteine residue on the synthetic immunogen peptides provides a free sulfhydryl group for covalent coupling to a maleimide-activated protein. A heterobifunctional reagent, such as an N-maleimido-6-aminocaproyl ester or a m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), is used to covalently couple the synthetic immunogenic peptide to the carrier protein (see for example, Hartlow, E. et al., Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 1988). Commercial kits are also readily available for use in coupling peptide antigens to maleimide-activated large carrier proteins.

Monoclonal antibodies may be obtained by methods known to those skilled in the art. See, for example Kohler and Milstein, 1975; U.S. Patent No. 4,376,110; Ausubel et al., eds., Current Protocols in Molecular Biology, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1987, 1992); and Harlow et al., supra; Colligan et al., eds., Current Protocols in Immunology, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1992-1997), the contents of which references are incorporated entirely herein by reference.

Once monoclonal antibodies are generated, the selectivity and binding affinity (Kd) can be evaluated by ELISA, and in vitro bioassays can be performed on the antibodies to test for the efficacy of the Aβ end-specific monoclonal antibodies in blocking Aβ aggregation and Aβ-induced cytotoxicity as described below in Example 1. Preferably, these monoclonal antibodies have not only a selectivity that is end-specific for specific Aβ peptides, but also have a high binding affinity. It is intended that the DNA encoding any antibody that is end-specific for the N-

terminus or C-terminus of Aβ peptides and exhibits efficacy
in blocking Aβ aggregation and Aβ induced cytotoxicity as
described in Example 1 can be used in generating the
recombinant antisenilin-encoding DNA molecules for use

5   according to the present invention. For instance, the C-
terminal end-specific monoclonal antibodies disclosed in WO
96/25435 may be used to obtain the recombinant antisenilin-
encoding DNA molecules according to the present invention.

Messenger RNA (mRNA) may then be isolated from

10  hybridomas producing Aβ end-specific monoclonal antibodies
determined to be selective for the free N-terminus or free C-
terminus of Aβ peptides. From the isolated hybridoma mRNA,
cDNA is synthesized and the nucleotide sequence encoding the
variable domains of the Aβ end-specific monoclonal antibody

15  may then be cloned using the polymerase chain reaction (PCR)
with primers based on the conserved sequences at each end of
the nucleotide sequences encoding the V domains of
immunoglobulin heavy chain ($V_H$) and light-chain ($V_L$). The
presence of restrictions sites incorporated into the sequence

20  of the PCR primers facilitates the cloning of PCR amplified
products encoding the variable region of the appropriate
chain.

A recombinant gene encoding a recombinant single
chain Fv antibody molecule is constructed, for example, by

25  joining nucleotide sequences encoding the $V_H$ and $V_L$ domains
with a nucleotide sequence encoding a peptide interchain
linker (Biocca et al., 1993; Duan et al., 1994; Mhashilkar et
al., 1995; Marasco et al., 1993; Richardson et al., 1995;
U.S. Patent 4,946,778; U.S. Patent 5,091,513, U.S. Patent

30  5,096,815) or by inserting the variable domain-encoding
nucleotide sequences to replace the corresponding sequences
encoding the variable domain in a human immunoglobulin gene
to thereby encode for a recombinant chimeric antibody
(Orlandi et al., 1989; U.S. Patent 4,816,567).

35        Standard reference works setting forth the general
principles of recombinant DNA technology include Ausubel et
al., eds., Current Protocols In Molecular Biology, Green
Publishing Assoc. and Wiley Interscience, N.Y. (1987-1997),

Watson *et al.*, <u>Molecular Biology of the Gene</u>, Volumes I and
II, The Benjamin/Cummings Publishing Company, Inc.,
publisher, Menlo Park, CA (1987); Darnell *et al.*, <u>Molecular</u>
<u>Cell Biology</u>, Scientific American Books, Inc., publisher, New

5   York, N.Y. (1986); Lewin, <u>Genes II</u>, John Wiley & Sons,
publishers, New York, N.Y. (1985); Old *et al.*, <u>Principles of</u>
<u>Gene Manipulation: An Introduction to Genetic Engineering</u>, 2d
edition, University of California Press, publisher, Berkeley,
CA (1981); Maniatis *et al.*, <u>Molecular Cloning: A Laboratory</u>

10  <u>Manual</u>, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
(1989); and Berger et al., <u>Guide to Molecular Cloning</u>
<u>Techniques</u>, Methods of Enzymology vo. 152, 1987, Academic
Press, Inc. San Diego, CA. These references are hereby
incorporated by reference.

15          The recombinant DNA molecule according to the
present invention, which contains a recombinant antibody
(antisenilin) gene, preferably also contains a promoter
operably linked to the recombinant antisenilin gene and
capable of expressing the antisenilin molecule in brain

20  cells. It will also be appreciated that, in order to
facilitate the secretion of the antisenilin molecule from
transformed cells expressing antisenilin, a leader or signal
peptide at the N-terminus is also provided.
          A DNA molecule is said to be "capable of

25  expressing" a polypeptide, such as the antisenilin molecule,
if it contains nucleotide sequences which contain
transcriptional and translational regulatory information, and
such sequences are "operably linked" to nucleotide sequences
which encode the polypeptide. An operable linkage is a

30  linkage in which the regulatory DNA sequences and the DNA
sequence sought to be expressed are connected in such a way
as to permit gene expression. The regulatory regions needed
for gene expression in general include a promoter region as
well as the DNA sequences which, when transcribed into RNA,

35  will signal the initiation of protein synthesis. Such
regions will normally include those 5'-non-coding sequences
involved with initiation of transcription and translation.

A promoter region would be operably linked to a DNA
sequence if the promoter were capable of effecting
transcription of that DNA sequence.  As used herein, a
"promoter sequence" is the sequence of the promoter which is
found on the DNA and is transcribed by the RNA polymerase.
Thus, to express antisenilins, transcriptional and
translational signals recognized by the host cell are
necessary.

The present method for preventing or inhibiting the
progression of Alzheimer's Disease involves administering to
a patient in need thereof a composition comprising a
recombinant DNA molecule in association with means for gene
delivery into cells of the central nervous system.  The
recombinant DNA molecule carries a gene encoding an
antisenilin molecule operably-linked to a promoter where this
operable linkage enables the expression of antisenilin
molecules in the brain.  The promoter is preferably a
promoter which would follow the expression pattern of βAPP
with the highest level of expression in the hippocampus and
cerebral cortex where amyloid deposition is most prevalent in
Alzheimer's Disease.  As a non-limiting example of a
preferred promoter operably linked to the antisenilin gene,
the thymidine kinase (Thy1) promoter has been shown to drive
the expression of βAPP in a region-specific manner that
mimics the natural expression of βAPP in the brain (Andra et
al., 1996).  Synapsin I promoter-based chimeric transgenes
have been used to target expression of βAPP in the CA
subfields of the hippocampus and in the piriform cortex in
brains of transgenic mice (Howland et al., 1995).  A high
level of βAPP expression has been achieved in brain cortex of
transgenic mice using a prion protein promoter (Hsiao et al.,
1996).  A number of advantages would be provided by using the
βAPP gene promoter to express the antisenilin gene.  In
particular, the antisenilin gene under the control of the
βAPP promoter would have identical anatomical and
physiological patterns of expression as the βAPP gene.  The
human βAPP promoter has been characterized by a number of
groups (e.g. Salbaum et al., 1988; La Fauci et al., 1989;

Wirak et al., 1991; Lahiri and Nall, 1995). The promoter has several regulatory domains including a heat-shock element and consensus sequences for the binding of transcription factors. Thus, expression of antisenilins under the control of the

5    βAPP gene can be enhanced as necessary in specific regions of the brain by applying any of a number of inducing agents, for example, growth factors, retinoic acid, and interleukin-1. A preproenkephalin promoter has also been reported to yield region-specific and long term expression in an adult rat

10   brain after direct *in vivo* gene transfer (Kaplitt et al., 1994).

In order to facilitate the introduction of a recombinant DNA molecule carrying an antisenilin gene operably-linked to a promoter into cells of the central

15   nervous system, a number of different means for gene delivery can be used in association with the recombinant DNA molecule. The term "means for gene delivery" is meant to include any technique suitable for delivery of DNA molecules across the blood brain barrier and/or for transmembrane delivery across

20   cell membranes. Non-limiting examples of the means for gene delivery are viral vectors (e.g., adeno-associated virus-based vectors), lipids/liposomes, ligands for cell surface receptors, etc.

The recombinant DNA molecule carrying the

25   antisenilin gene is associated with the means for gene delivery where such association is intended to encompass, for example, the situation in which the means for gene delivery in a viral vector and the antisenilin gene is incorporated in the DNA of the viral vector or packaged in the viral

30   particle; the situation in which the means for gene delivery is a liposome and the antisenilin gene is complexed therewith; the situation in which the means for gene delivery is a ligand for a cell surface receptor and the antisenilin gene is conjugated or otherwise bound thereto; etc. Thus,

35   "in association with" includes incorporating or packaging in, complexing with, conjugating or binding to, and any other manner of associating the antisenilin gene with the means for gene delivery. It will be appreciated that the recombinant

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DNA molecule may be in association with more than one means
for gene delivery, particularly where the recombinant DNA
molecule is to be delivered across both the blood brain
barrier and the cell membrane of brain cells.

5            Adeno-associated virus (AAV) was initially isolated
as a tissue culture contaminant and was later found as a non-
pathogenic coinfecting agent during an adenovirus outbreak in
children (Blacklow et al., 1968).  It is a single-stranded
DNA virus of the parvovirus group with a 4.7 kb genome.  As
10   one of the smallest human DNA viruses, AAV requires
coinfection with a helper virus, usually an adenovirus or
herpesvirus, for efficient replication in order to complete
its life cycle (Carter, 1990).  In the absence of helper
virus infection, AAV becomes latent and stably integrates at
15   high frequency, often at a specific site on chromosome 19
(Kotin et al., 1990; 1991; 1992; Samulski et al., 1991).  The
AAV genome has been sequenced and it was discovered that the
sole sequence needed for integration of an AAV vector is in
the terminal 145 nucleotide inverted terminal repeats (ITR),
20   thus making the cloning capacity nearly 4.7 kb (Muzyczka,
1992).  Due to the non-pathogenic nature of the virus, its
broad host cell range, and its ability to take advantage of a
natural mechanism for high frequency integration, AAV is
particularly suitable as a vector for gene delivery/transfer
25   into cells.         Moreover, while conventional retroviruses
have a requirement for genomic DNA synthesis, AAV vectors
have a unique ability to introduce foreign genes into non-
dividing or quiescent cells.  These characteristics are being
increasingly exploited for gene expression in the mammalian
30   brain, and several genes related to Alzheimer's Disease have
been expressed in the brain using AAV vectors (Makimura et
al., 1996).  Recent studies by Du et al., 1996, indicate that
AAV vectors can efficiently transduce and stably express a
foreign gene, e.g., *lacZ*, in post-mitotic human neurons.  The
35   expression of foreign genes in neuronal cells has also been
reported using liposome-mediated transfection with AAV-
derived plasmids (Meyer et al., 1995; Wu et al., 1994, 1995).

Low et al., U.S. Patent 5,108,921, reviews available methods for transmembrane delivery of molecules such as proteins and nucleic acids by the mechanism of receptor mediated endocytotic activity. These receptor
5    systems include those recognizing galactose, mannose, mannose-6-phosphate, transferrin, asialoglycoprotein, transcobalamin (vitamin $B_{12}$), $\alpha$-2 macroglobulins, insulin and other peptide growth factors such epidermal growth factor (EGF). Low et al. also teaches that nutrient receptors, such
10   as receptors for biotin and folate, can be advantageously used to enhance transport across the cell membrane due to the location and multiplicity of biotin and folate receptors on the membrane surfaces of most cells, and the associated receptor mediated transmembrane transport processes. Thus, a
15   complex formed between a compound to be delivered into the cytoplasm and a ligand, such as biotin or folate, is contacted with a cell membrane bearing biotin or folate receptors to initiate the receptor mediated trans-membrane transport mechanism and thereby permit entry of the desired
20   compound into the cell.

A biotin ligand can be attached to a DNA molecule, for example, by incorporating commercially available biotinylated deoxynucleotide triphosphates, e.g., biotin-14-dATP or biotin-14-dCTP from Life Technologies, Inc.,
25   Gaithersburg, MD, using terminal deoxynucleotidyl transferase (Karger, B.D., 1989). Biotin-14-dATP is a dATP analog with biotin attached at the 6-position of the purine base by a 14-atom linker and biotin-14-dCTP is a dCTP analog with biotin attached at the $N^4$-position of the pyrimidine base also by a
30   14-atom linker.

Whether incorporated into a viral-based or plasmid vector for packaging into a virus, attached to a neural receptor-binding ligand molecule, complexed with cationic lipids or cationic liposomes, or in association with other
35   suitable means for gene delivery, the recombinant DNA molecule encoding an antisenilin gene operably linked to a promoter is administered to a subject by injection. Stereotactic microinjection into different brain regions

- 20 -

through use of established coordinates can be used to deliver the viral packaged or ligand-bound recombinant DNA molecule directly into the extracellular environment, e.g., cerebrospinal fluid, surrounding brain cells for subsequent

5    transmembrane delivery into the cells themselves.

As direct injection into the brain is an invasive procedure, it is preferred that the viral packaged or ligand-bound recombinant DNA molecule be administered by intravenous or intra-arterial injection. The viral packaged or ligand-

10   bound recombinant DNA can further be in association with other means for gene delivery, such as to effect gene delivery across the blood-brain barrier into the central nervous system. Zhu et al., 1993, demonstrated that cationic lipid-plasmid DNA complexes can be delivered systemically to

15   all tissues including the brain. Recently, it has also been shown that intra-arterially administered cationic liposomes containing the thymidine kinase gene was successful in a rat model of brain tumor where regression was achieved without apparent toxicity or histological damage (Laine et al.,

20   1995). Gene delivery by liposomes is well covered in the scientific literature and in patent publications, and extensively reviewed by Lasic, D.D., In:Liposomes in Gene Delivery, CRC Press, Boca Raton, Florida, 1997, which is hereby incorporated entirely by reference.

25   Once delivered to the brain, the viral packaged recombinant DNA molecule, either ligand-bound or in association with another suitable means for gene delivery, transforms brain cells, which subsequently express antisenilin molecules (recombinant antibody molecules end-

30   specific for A$\beta$ peptides) and secrete the expressed antisenilins into the extracellular space, interstitial fluid and cerebrospinal fluid. The secreted antisenilins then form a soluble complex with A$\beta$ peptide to which they are end-specific in the extracellular space, interstitial fluid and

35   cerebrospinal fluid. These soluble antisenilin-A$\beta$ peptide complexes prevent the aggregation of A$\beta$ peptides into amyloid deposits and prevent A$\beta$-induced neurotoxicity by clearing A$\beta$ peptides from the central nervous system through drainage of

the extracellular space, interstitial fluid and cerebrospinal
fluid into the general blood circulation where they will be
eliminated by protease digestion.  Accordingly, the
accumulation of newly-secreted soluble A$\beta$ peptides
5    responsible for amyloid deposition and A$\beta$-induced
neurotoxicity is prevented.

While the present method for preventing or
inhibiting the progression of Alzheimer's Disease is intended
to be primarily used for patients with a clear genetic
10   disposition to developing Alzheimer's Disease, it can also be
used prophylactically to "immunize" the population in general
against the occurrence of such a prevalent and debilitating
disease.  The preferred route of administration is
intravenous or intra-arterial.  However, despite the
15   invasiveness of microinjection directly into regions of the
brain, this route of administration is intended to be within
the scope of the invention.  In particular, patients having
Down's Syndrome or familial Alzheimer's Disease-linked
mutations who are expected to develop Alzheimer's Disease due
20   their predisposition or patients who already suffer from
Alzheimer's Disease can be treated by direct microinjection
into the brain.  The benefit of this treatment is expected to
outweigh the risks of an invasive technique such as injection
into the brain.

25   The recombinant DNA molecule which contains an
antisenilin gene in association with a means for gene
delivery may be used in the preparation or manufacture of a
medicament/pharmaceutical composition.  The pharmaceutical
compositions contain an amount of the recombinant DNA
30   molecule effective to achieve its intended purpose.  For
instance, when the means for gene delivery is a viral vector,
such as an AAV vector, a suitable dosage of viral particles
in a pharmaceutical composition to be stereotactically
microinjected into different locations in the brain is in the
35   range of about $5 \times 10^4$ to $1 \times 10^{11}$ particles.  When a ligand, such
as biotin, is used as the means for gene delivery by
administration directly into the brain, ligand-bound DNA
molecules in the range of about 0.5 to 100 $\mu$g are suitably

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used.  For such ligand bound DNA molecules, it is preferred
that the DNA molecules are condensed beforehand to protect
these molecules in the extracellular milieu of cells within
the central nervous system.  Pharmaceutical compositions and
5   dosages of DNA molecules complexed with cationic lipids or
cationic liposomes are discussed in Lasic, 1997, *supra*.
Furthermore, the pharmaceutical compositions may contain
suitable pharmaceutically acceptable excipients, such as are
well-known in the art.

10          Having now generally described the invention, the
same will be more readily understood through reference to the
following prophetic example, which is provided by way of
illustration and is not intended to be limiting of the
present invention.

15

EXAMPLE 1

            The strategy and the protocols for use in
developing recombinant DNA molecules containing a gene
encoding a recombinant antisenilin antibody molecule end-
20  specific for an amyloid-$\beta$ peptide are described below.


**MONOCLONAL A$\beta$ END-SPECIFIC ANTIBODY PRODUCTION**
**Immunogen peptide synthesis**

            Several peptides of varying lengths incorporating
25  either the free N-terminus or free C-terminus are prepared
using an Applied Biosystems Peptide Synthesizer (430A).  The
synthetic peptides are purified by HPLC and characterized
using both amino acid composition and $NH_2$-terminal micro
sequence analyses.

30

<u>Peptide N1/5 A$\beta_{1-40/42}$  (mAb:  "Antisenilin N1/5")</u>

            A peptide corresponding to the first five amino
acid residues of A$\beta$ (1-40 and 1-42), as schematically
represented by the appropriate line segment in Fig. 2, is
35  synthesized.  The peptide contains a cysteine residue at the
C terminus and has the sequence of SEQ ID NO:2 (See Figure
1).

Peptide N1/7Aβ$_{1-40/42}$ (mAb: "Antisenilin N1/7")

   A peptide corresponding to the first seven amino acid residues of Aβ (1-40 and 1-42), as schematically represented by the appropriate line segment in Fig. 2, are
5 synthesized. The peptide contains a cysteine residue at the C terminus and has the sequence of SEQ ID NO:3.


Peptide C34/40Aβ$_{1-40}$ (mAb: "Antisenilin C34/40")

   A peptide corresponding to the last seven amino
10 acid residues of Aβ (1-40), as schematically represented by the appropriate line segment in Fig. 2, is synthesized. The peptide contains a cysteine residue at the N-terminus and has the sequence of SEQ ID NO:4.


15 Peptide C36/42Aβ$_{1-42}$ (MAb: "Antisenilin C36/42")

   A peptide corresponding to the last seven amino acid residues of Aβ (1-42), as schematically represented by the appropriate line segment in Fig. 2, is synthesized. The peptide contains a cysteine residue at the N-terminus and has
20 the sequence of SEQ ID NO:5.


**Peptide conjugation**

   The purified peptides are conjugated to bovine serum albumin (BSA) using N-maleimido-6-aminocaproyl ester of
25 1-hydroxyl-2-nitro-4-benzene-sulfonic acid.


**Immunization and hybridoma monoclonal antibody production**
Phase 1: Four sets of 10 Balb/c mice are immunized with the purified BSA-conjugated peptides described above using
30 standard immunization protocols (Taggert and Samloff, 1983).
Phase 2: Following the completion of the immunization protocol, a fusion procedure is performed using spleenoxytes from the hyperimmunized mice and an appropriate myeloma cell-line SP2/0-Ag14 (ATCC CRL 1581), NS-1 (ATCC TIB18), or
35 equivalent. This procedure is performed using polyethylene glycol, and the selection of successful fusion products are achieved by means of HAT media. Viable hybridoma colonies are grown out in 96 well plates.

Phase 3: Screening of all wells containing successful fusion products are carried out using ELISA described in the next section with the peptide antigens. Supernatants from several wells are also screened in the *in vitro* bioassays as

5    described below.

Phase 4: On the basis of the results of ELISA assays and the evaluations based the results of the bioassays, subcloning is performed by limiting dilutions on the selected colonies.

10   **ELISA detection and affinity determinations**

The specificity and binding affinities (Kds) of the monoclonal antibodies are evaluated by ELISA assays (Engvall and Perlmann, 1971) using a set of synthetic peptides corresponding to Aβ 1-42, Aβ 1-40, and residues 1-52, 1-11, -

15   2[KM]-11, -1[M]-11, 1-28, 35-40, 35-42, and 35-44 found in Aβ peptides and βAPP from which they are derived. In addition, the immunogenic peptide sequences, corresponding to the N-terminus or C-terminus of Aβ peptides, and conjugated to a different carrier protein, such as keyhole limpet hemocyanin

20   (KLH) and ovalbumin, are used to determine whether the resultant monoclonal antibodies are end-specific for Aβ peptides and non-specific for the carrier protein or the cysteine bridge.

To test the protocol to be used subsequently to

25   generate monoclonal antibodies, high affinity polyclonal antibodies specific for the free N-terminus of Aβ peptides were made where the antibodies were raised using the restricted peptide: $H_2N$ - SEQ ID NO:6 - aminohexanoate-C-amide. The peptides were synthesized using solid phase Fmoc

30   chemistry. The peptides were then cleaved and analyzed by mass spectroscopy and high performance liquid chromatography (HPLC). HPLC purification was achieved using a C-18 YMC column (10 μ packing, 120 A pore size, 10 X 250 mm) in a buffer system of A: $H_2O$/0.1% TFA and B:$CH_3CN$/0.08% TFA. The

35   appropriate fractions were pooled, lyophilized, and again subjected to mass spectroscopy and HPLC analysis. The peptide was coupled to KLH for immunization, BSA for ELISA detection, with the cross-linker MBS. Rabbits were immunized

at 3 .week intervals, and the titer assessed by ELISA using
acetal-SEQ ID NO:7-Ahx-C-amide.  This peptide corresponds to
a sequence of amino acid residues that spans the 0 to 1
splice site that yields the free N-terminus of Aβ peptides.

5   The same spanning peptide was coupled to a thiol coupling gel
via their cysteine residue and used to preabsorb away all
antibodies which do not depend upon the free amine-Asp being
present.  The antibodies were then purified and collected
using the N-terminal peptide.  Whereas the crude serum shows

10  substantial activity towards the spanning peptide, once
affinity purified, there is no reactivity of the resulting
antibody with the spanning peptide, only with the N-terminal
peptide.

        To generate monoclonal antibodies specific for the

.15  N-terminus of the Aβ peptides, mice are immunized at 3 week
intervals using: $H_2N$-SEQ ID NO:6-aminohexanoate-C-amide
conjugated to BSA prepared as described for the preparation
of polyclonal.  The titer in each mouse is also assessed by
ELISA as described above.  After spleen cell fusion of the

20   mice containing the highest titer, several clones are
isolated and screened using the spanning peptide ELISA
detection method.


**In vitro bioassays to test efficacy of Aβ end-specific**
25  **antibodies in blocking Aβ aggregation and Aβ-induced**
**cytotoxicity**


A) **Effects on Aβ Fibril Formation:**  As shown by Jarrett et
al. (1993), the carboxyterminus of Aβ is critical for the
30   "seeding" of amyloid formation which is probably responsible
for the greatly accelerated rate of amyloid plaque formation
in Alzheimer's Disease (Yankner and Mesulam, 1991).  Amyloid
formation by the kinetically soluble peptides, such as Aβ 1-
40, can be nucleated or "seeded" by peptides such as Aβ 1-42

35   that include the critical C-terminal residues 41(Ile) and
42(Ala).  After the April 9, 1997, filing date of the
provisional U.S. application on which the present application
claims benefit of priority, abstracts by Solomon et al.

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(1997) and Frenkel et al. (1997) reported that their studies
show that antibodies directed towards the N-terminal region
of positions 1-16 of Aβ peptides bind to formed fibrils and
lead to disaggregation. The anti-aggregating epitope of such

5   an antibody is reported to be located within just the four
amino acids Glu Phe Arg His (SEQ ID NO:8) of residue
positions 3-6. These four amino acid residues of SEQ ID NO:8
are all present in the immunizing peptides for the N-terminus
of Aβ. The ability of C-terminus or N-terminus end-specific

10  Aβ antibodies to block seeding by Aβ 1-42 or to prevent
aggregation of amyloid peptides is tested using standard
aggregation assays (Wood et al., 1996). The Aβ 1-40 peptide
is solubilized to 5 mg/ml in 1,1,1,3,3,3-hexafluoro-2-
propanol. The peptide is concentrated to dryness and

15  resolubilized in phosphate-buffered saline (PBS), pH 7.4, to
a final concentration of 230 $\mu$M. A solution of Aβ 1-42 (20
$\mu$M) is stirred for 3 days and sonicated for 30 min to produce
amyloid fibrils. Preaggregated Aβ1-42 at 2nM concentration
is added to the supersaturated pH 7.4 incubation to seed

20  aggregation of Aβ 1-40. Aggregate formation in the absence
and in the presence of each Aβ end-specific monoclonal
antibody is determined by monitoring the turbidity of samples
prepared in microtiter wells using a microtiter plate reader
at 405 nm. The reaction is also monitored by thioflavin-T

25  fluorescence as described by Wood et al. (1996). The ability
of N-terminus-specific antibodies to promote disaggregation
of amyloid peptide fibrils is tested by testing the
displacement of [$^{125}$I]-labeled amyloid aggregated peptides
from a collagen matrix containing non-aggregated peptides

30  coated onto 96-well microtiter-plastic-coated plates. In
addition, the ability of N-terminus-specific-antibodies to
protect neurons from Aβ-induced damage is assessed by the
trypan blue exclusion method, intracellular calcium
measurements, scanning and transmission electron microscopy

35  and by confocal microscopy.


B) <u>Aβ-induced neurotoxicity:</u> The receptor for advanced
glycation end products (RAGE) mediates some of the neurotoxic

effects of Aβ on neurons and microglia (Yan et al., 1996).
End-specific antibodies are tested for their ability to
inhibit the receptor-mediated neurotoxicity by competitive
inhibition.  The antibodies are tested both with purified
5   RAGE receptor preparations and by measuring their effect on
Aβ-induced cellular oxidant stress.

The RAGE receptor is purified from a bovine lung
extract dissolved in tris-buffered saline containing octyl-β-
glucoside (1%) and phenylmethylsulfonylfluoride (2 nm) and
10  applied to a heparin hyperD column (Biosepra).  The column is
eluted with a gradient of NaCl and fractions with maximal
binding of $^{125}$I-labeled Aβ are identified.  The fractions are
pooled and loaded onto hydroxyapatite ultragel (Biosepra) and
eluted with increasing concentrations of phosphate.
15  Fractions with maximal binding of $^{125}$I-labeled Aβ are applied
to preparative non-reduced polyacrylamide SDS gels (10%).
The RAGE receptor protein $M_r$ 50,000 is identified by
Coommassie Blue staining and the region in adjacent lanes are
cut and eluted.  Competitive inhibition by the end-specific
20  antibodies to binding of $^{125}$I-labeled Aβ(1-40/1-42) to the
RAGE receptor is determined in a number of ways: (1)
different amounts (0-150 μg) of purified protein are
immobilized on microtiter wells and incubated with 100 nM
$^{125}$I-labeled Aβ(1-40/1-42); (2) different amounts (0-250 nM)
25  of $^{125}$I-labeled Aβ(1-40/1-42) are incubated in microtiter
wells pre-coated with 5 μg purified RAGE receptor; and (3)
different amounts (0-500 ug/ml) of Aβ(1-40/1-42) are
immobilized on microtiter wells and incubated with 50 nM
$^{125}$I-labeled RAGE receptor.  In each assay, the amount of
30  ligand binding to the well in the presence of different
amounts of antibody is determined by counting the amount of
radioactivity in the wells with a gamma-scintillation
counter.

To evaluate the efficacy of the different
35  end-specific Aβ monoclonal antibodies as inhibitors of
Aβ-induced cellular oxidant stress, cultured mouse brain
microvascular endothelial cells (Breitner et al., 1994) are
incubated with 0.25 μM Aβ in the presence of different

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amounts of the antibodies, and cellular oxidant stress is
assessed by measuring the dose-dependent generation of
thiobarbituric acid-reactive substances using the TBARS assay
as previously described (Dennery et al., 1990; Yan et al.,

5     1996). In a parallel assay system (developed by Khoury et
al., 1996), the inhibitory effects of the antibodies are
tested on Aβ-induced production of oxygen-reactive species in
N9 mouse microglial cells. N9 cells (5 x $10^{4}$) are incubated
at 37°C in the presence of different amounts of the

10    antibodies in 50 μl PD-BSA (phosphate-buffered saline lacking
divalent cation having 1 mg/ml BSA) containing 1 μM $H_2$DCF
(2',7'-dichlorofluorescein diacetate), a dye that fluoresces
upon oxidation (Wan et al., 1993) on multispot slides coated
with Aβ peptides. At various time points, aliquots of the

15    culture medium are taken and the fluorescence is measured in
a fluorescence plate reader (Cytofluor II).


C) **Effect on interactions with proteoglycans:** The vascular
cell derived heparan sulfate proteoglycan, perlecan, has been

20    identified in all amyloid deposits and is implicated in the
earliest stages of inflammation-associated amyloid induction
through high-affinity binding interactions with Aβ (Snow et
al. 1989; 1995). Binding of perlecan to Aβ imparts secondary
and tertiary amyloid structural features which suggest that

25    molecules that interfere with the interaction may prevent or
arrest amyloidogenesis.

      End-specific Aβ monoclonal antibodies made to
peptides of different lengths that correspond to the
N-terminus of the peptide are evaluated for their ability to

30    block the binding of perlecan to the perlecan binding site in
the N-terminus region of Aβ (Snow et al., 1995). These
evaluations are based on a solid-phase binding assay using
perlecan isolated from cultured endothelial cells prepared
from calf thoracic aortas as described in detail by (Snow et

35    al. 1995). Polyvinyl micro-titer wells are coated with 100
μl of nitrocellulose solution and allowed to dry. Wells are
then coated overnight at room temp with unlabeled perlecan to
give 0.28 ug of bound perlecan per well, and blocked

overnight at room temp with 200 μl of 5% non-fat dried milk.
Various quantities of $^{125}I$ Aβ (7000 cpm/pM) diluted in 100 μl
of TBS/0.05% Tween 20 (TBST) are added in triplicate to the
wells and incubated for 2.5 h at room temp on an orbital

5     shaker.  At the end of the incubation period, free $^{125}I$ Aβ is
removed with six washes of TBST.  Bound $^{125}I$ is extracted in
100 μl 1N sodium hydroxide and "bound" versus "free"
radioactivity is quantitated by liquid scintillation
counting.  Scatchard analysis is performed after incubating

10    $^{125}I$-Aβ in the presence of increasing amounts of monoclonal
antibody.


## CLONING AND ASSEMBLY OF RECOMBINANT GENES
### mRNA isolation and cDNA synthesis from hybridomas

15            Messenger RNA (mRNA) is prepared from $5 \times 10^8$
hybridoma cells as described by Griffiths and Milstein
(1985).  First-strand cDNA synthesis is performed according
to standard procedures (Maniatis et al., 1989).


20    ## PCR amplification, cloning of variable antigen-binding region
### and construction of single-chain antibodies


            Techniques have been developed for the cloning of
immunoglobulin variable domains from genomic DNA and cDNA

25    using the polymerase chain reaction (Orlandi et al., 1989;
Ward et al., 1989; Richardson et al., 1995).  Primers based
on conserved sequences at each end of the nucleotide
sequences encoding V domains of mouse immunoglobulin
heavy-chain ($V_H$) and kappa light-chain ($V_K$) also incorporate

30    restriction sites that permit force-cloning of the amplified
product containing the variable region of each chain.  These
primers are capable of amplifying most immunoglobulin mRNA of
the mouse repertoire.
            As shown in Fig. 4, PCR on cDNA from the hybridoma

35    cells is performed using the primers described by Richardson
et al., 1995.  The scFv antisenilin gene is assembled from
the amplified DNA corresponding to the $V_H$ and $V_L$ regions and
an interchain linker, expressed in E. coli, and reamplified

by PCR using primers that incorporates a stop codon at the 3'-end of $V_L$ as described by Richardson et al. 1995. To prepare for the construction of a recombinant AAV vector, an XbaI restriction site is incorporated into the forward and
5    reverse primers for reamplifying the recombinant scFv gene so as to facilitate its insertion into the AAV plasmid vector pSSV discussed immediately below.


## Construction of recombinant adeno associated viral vectors
10  ## for regional expression of scFvαAβ genes in the brain

The assembled ScFvαAβ genes are ligated into an AAV plasmid pSSV9 (psub201) under the control of the human βAPP promoter (huβAPPP). Plasmid pSSV9 is a modified full-length AAV type 2 genomic clone. Alternatively, other suitable
15  promoters, such as Thy-1, synapsin I, prion, etc. can be used as discussed previously, although huβAPPP is preferred.

As shown in Figure 5, all of the AAV coding sequences are excised, leaving only the viral inverted terminal repeats (ITR) by cleavage of the two flanking XbaI
20  sites. These ITRs contain the recognition signals necessary for replication and packaging into an AAV vector. The AAV coding sequences are replaced with the hu $\beta APPP_{H/K}A\beta$ coding sequences. The new coding sequences of AAV/hu$\beta APPP_{H/K}A\beta$ are followed by an SV40 early region polyadenylation signal.
25


## Preparation of packaged hu $\beta APPP_{H/K}A\beta AAV$ vectors

hu$\beta APPP_{H/K}A\beta$ AAV vectors are packaged by co-complementation as described by Samulski et al., 1989, using an adenovirus-transformed human embryonic kidney cell line,
30  293 (ATCC CRL-1573). The cells are cultured in Eagle's MEM supplemented with glutamine and Earle's salts and 10% heat-inactivated fetal calf serum at 37°C in a humidified incubator with 5% $CO_2$. Adenovirus type 5 (Ad5) stocks are raised by 1 h infection of subconfluent 293 cells with 10 $\mu$l
35  of Ad5 seed culture in 1 ml of serum-free DMEM per 100 mm dish. After 48 to 72 h, when strong cytopathic effects are observed, the cells are collected and pelleted by centrifugation. The cells are lysed by freeze/thaw cycles to

- 31 -

release the intracellular virus and debris is removed by low-speed centrifugation. Virus containing supernatants are aliquoted and stored -70°C. Co-complementation is accomplished as follows: Semi-confluent 293 cells plated at

5   $0.5 \times 10^6$ cells per 100 mm plate are infected with Ad5 at a multiplicity of infection of 10. After 1 h, the cells are co-transfected with 20 $\mu$g of hu$\beta$APPP$_{H/K}\alpha$A$\beta$ AAV plasmids and 10 $\mu$g of pAd8 which contains the AAV 2 genes encoding the AAV replication and encapsidation functions, but flanked by

10   terminal repeats that are derived from adenovirus 2, rather than AAV (Samulski et al., 1987, 1989). Transfection is performed using a standard calcium phosphate precipitation method (Wigler et al., 1979) with the addition of chloroquine diphosphate to enhance the transfection efficiency (Luthman

15   et al., 1983). After overnight incubation, the transfection solution is replaced with fresh medium containing 15% fetal calf serum. Three days after infection the cells are harvested, pelleted by centrifugation at 1000 x g for 10 min, and then subjected to freeze thaw cycles to release the

20   cell-associated virons. Contaminating Ad5 is inactivated by heating lysates to 56°C. The lysates are then clarified by centrifugation and treated with 25 units/ml of RNASE-free DNASE at 37°C for 30 min to remove any remaining plasmid DNA.

25

**Transduction of human neurons to test expression of AAV/hu$\beta$APPP$_{H/K}$A$\beta$ vectors**

The utility of AAV as a potential vector has been

30   established unequivocally by Du et al., 1996, in human NT neurons (Pleasure et al., 1993). The precursor of these neurons is a subline of human teratocarcinoma cells, NT2, that commits to terminal differentiation into neurons on exposure to retinoic acid (Lee et al., 1994; Pleasure et al.,

35   1993). Four weeks of retinoic acid treatment accompanied by selective replatings can yield nearly pure > 95 % populations of neurons. These mature neurons remain viable in culture for many weeks. In addition to the distinct morphological

appearance and expression of many neuronal markers, human NT
neurons have similar patterns of amyloid precursor proteins
as native CNS neurons and produce A$\beta$ peptides (Wertkin et
al., 1993).

5          Undifferentiated precursor NT2 cells are obtained
from Stratagene, La Jolla, CA, and cultured in Opti-MEM
(GIBCO BRL, Gaithersburg, MD) containing 5% heat-inactivated
fetal bovine serum (FBS) and 100 units/ml penicillin and 100
$\mu$g/ml streptomycin (PS) at 37°C in 5% $CO_2$.  2 x $10^6$ NT2 cells
10   per T75 flask are treated with 10 $\mu$M retinoic acid for four
weeks and then replated at low density into six T75 flasks.
The top layers containing differentiated cells are
mechanically dislodged and replated at 1 x $10^6$ cells per well
in 24-well plates.  Wells and glass cover-slips are coated
15   with 0.01% poly-D-lysine followed by 1:20 MATRIGEL
(Collaborative Research, Bedford, MA).  Cells are cultured in
DMEM high glucose/L-glutamine containing 10% FBS, PS and
mitotic inhibitors for three weeks.  The enriched neurons are
maintained in DMEM/10% FBS/PS at 37°C, 5% $CO_2$.
20          NT neurons (about $10^5$ per well) are transduced with
AAV/hu$\beta$APPP$_{H/K}$A$\beta$ vectors by removing the growth medium,
washing once with serum-free medium and adding vector stock
diluted in serum-free DMEM.  After incubating for 90 min at
37°C, 1 ml of DMEM containing 10% FBS is added to each well.
25   The cultures receive a change of medium after two days, and
twice weekly thereafter.


Cell viability assay

          The viability of cells is evaluated on the basis of
30   the mitochondrial function of control and hu$\beta$APPPV$_{H/K}$A$\beta$ AAV
vector transduced cells.  The levels of mitochondrial
dehydrogenase activity were compared using 3-
(4,5-Dimethylthiazol-3-yl)-2,5-diphenyl tetrazolium bromide
as the substrate.  Its cleavage to a purple formazan product
35   by dehydrogenase is spectophotometrically quantified at 570
nm.


Detection and determination of binding affinity constants

To verify that the secreted recombinant antibodies (antisenilins) retain the binding properties of the original hybridoma secreted antibodies, ELISA assays are performed, as described earlier in this example, with the culture medium of
5    NT2 transduced cells.


## Bioassays to test inhibition of Aβ functions

The secreted antibodies are isolated from the culture medium in which the transduced NT2 cells are
10   incubated.  The purified antibodies and the culture-medium itself are tested as inhibitors of Aβ aggregation or Aβ induced cytotoxicity as described above in the section on *in vitro* bioassays.

## Generation of transgenic mice expressing the single-chain
15   antisenilin antibodies in the brain


The ScFv antisenilin gene is inserted into a hamster prion protein (PrP) cosmid vector in which the PrP open reading frame (ORF) is replaced with the antisenilin
20   gene ORF.  The transgenes are used to generate transgenic mice by microinjection into fertilized 1-cell eggs of C57B6SJL mice according to any one of the widely used methods, such as Brinster et al. (1981), Harbers et al. (1981), Wagner et al. (1981), Gordon et al. (1976), Stewart
25   et al. (1982), Palmiter et al. (1983), and U.S. Patent No. 4,870,009.  The resulting progeny (TGScFvA) are tested by genotyping using standard PCR amplification procedures.


## Animal models to establish the therapeutic potential of the
30   αβ antibodies as antisenilins


Animal models are required to test for the expression of anti-Aβ antibodies *in vivo* and to determine whether they demonstrate a potential for slowing down the
35   accumulation of amyloid plaques and prevent tbe development of AD-like pathology in tbe brain.  Although AD is a uniquely human disease, a number of transgenic mice that overexpress human βAPP show promise.

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## Effects of chronic Aβ depletion on plaque burden and related AD pathology transgenic mice

The antisenilin function of the recombinant Aβ
5    end-specific antibodies are tested in a transgenic animal
mouse model that overexpresses the 695-amino acid isoform of
Alzheimer βAPP containing a Lys670 to Asn, Met671 to Leu
mutation (Hsiao, K., 1996, U.S. Patent Application no.
08/664,872). The correlative appearance of behavioral,
10   biochemical, and pathological abnormalities reminiscent of
Alzheimer's Disease in these transgenic mice (TG2576)
provides the opportunity to explore the usefulness of agents
to slow down or prevent the Aβ-induced pathophysiology of the
disease.

15       Female transgenic mice (TGScFvA) homozygous for the
antisenilin gene are crossed with breeding TG2576 males. The
offspring that express both the antisenilin gene and the
variant APP gene are compared with respect to behavioral,
biochemical, and pathological abnormalities with TG2576 mice.

20       Having now fully described this invention, it will
be appreciated by those skilled in the art that the same can
be performed within a wide range of equivalent parameters,
concentrations, and conditions without departing from the
spirit and scope of the invention and without undue
25   experimentation.

While this invention has been described in
connection with specific embodiments thereof, it will be
understood that it is capable of further modifications. This
application is intended to cover any variations, uses, or
30   adaptations of the inventions following, in general, the
principles of the invention and including such departures
from the present disclosure as come within known or customary
practice within the art to which the invention pertains and
as may be applied to the essential features hereinbefore set
35   forth as follows in the scope of the appended claims.

All references cited herein, including journal
articles or abstracts, published or unpublished U.S. or
foreign patent applications, issued U.S. or foreign patents,

or any other references, are entirely incorporated by
reference herein, including all data, tables, figures, and
text presented in the cited references. Additionally, the
entire contents of the references cited within the references
5   cited herein are also entirely incorporated by reference.

Reference to known method steps, conventional
methods steps, known methods or conventional methods is not
in any way an admission that any aspect, description or
embodiment of the present invention is disclosed, taught or
10  suggested in the relevant art.

The foregoing description of the specific
embodiments will so fully reveal the general nature of the
invention that others can, by applying knowledge within the
skill of the art (including the contents of the references
15  cited herein), readily modify and/or adapt for various
applications such specific embodiments, without undue
experimentation, without departing from the general concept
of the present invention. Therefore, such adaptations and
modifications are intended to be within the meaning and range
20  of equivalents of the disclosed embodiments, based on the
teaching and guidance presented herein. It is to be
understood that the phraseology or terminology herein is for
the purpose of description and not of limitation, such that
the terminology or phraseology of the present specification
25  is to be interpreted by the skilled artisan in light of the
teachings and guidance presented herein, in combination with
the knowledge of one of ordinary skill in the art.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

   (i) APPLICANT: CHAIN, Daniel G.

  (ii) TITLE OF INVENTION: DNA ENCODING RECOMBINANT ANTIBODY MOLECULES
       END-SPECIFIC FOR AMYLOID-BETA PEPTIDES, PHARMACEUTICAL
     COMPOSITIONS THEREOF, AND METHOD OF PREVENTING OR INHIBITING
     PROGRESSION OF ALZHEIMER'S DISEASE

 (iii) NUMBER OF SEQUENCES: 5

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     (F) ZIP: 20004

   (v) COMPUTER READABLE FORM:
     (A) MEDIUM TYPE: Floppy disk
     (B) COMPUTER: IBM PC compatible
     (C) OPERATING SYSTEM: PC-DOS/MS-DOS
     (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

  (vi) CURRENT APPLICATION DATA:
     (A) APPLICATION NUMBER:
     (B) FILING DATE:
     (C) CLASSIFICATION:

 (viii) ATTORNEY/AGENT INFORMATION:
     (A) NAME: YUN, Allen C.
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     (A) TELEPHONE: (202) 628-5197
     (B) TELEFAX: (202) 737-3528

(2) INFORMATION FOR SEQ ID NO:1:

   (i) SEQUENCE CHARACTERISTICS:
     (A) LENGTH: 59 amino acids
     (B) TYPE: amino acid
     (C) STRANDEDNESS: single
     (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: peptide

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
1               5                   10                  15

His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
            20                  25                  30

Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
            35                  40                  45

Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys
        50                  55
```

(2) INFORMATION FOR SEQ ID NO:2:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 6 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: cDNA

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

    Asp Ala Glu Phe Arg Cys
    1               5


(2) INFORMATION FOR SEQ ID NO:3:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

    Asp Ala Glu Phe Arg His Asp Cys
    1               5


(2) INFORMATION FOR SEQ ID NO:4:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

    Cys Leu Met Val Gly Gly Val Val
    1               5


(2) INFORMATION FOR SEQ ID NO:5:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 8 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

    Cys Val Gly Gly Val Val Ile Ala
    1               5

(2) INFORMATION FOR SEQ ID NO:6:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 6 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single

            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

    Asp Ala Glu Phe Arg His
    1               5

(2) INFORMATION FOR SEQ ID NO:7:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 13 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

    Glu Ile Ser Glu Val Lys Met Asp Ala Glu Phe Arg His
    1               5                   10

(2) INFORMATION FOR SEQ ID NO:8:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 4 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

    Glu Phe Arg His
    1

WHAT IS CLAIMED IS:

1.      A method for preventing or inhibiting
progression of Alzheimer's Disease, comprising the step of
administering a composition comprising a recombinant DNA
molecule, containing a gene encoding a recombinant antibody
molecule end-specific for the N-terminus or the C-terminus of
an amyloid-β peptide, operably-linked to a promoter which is
expressed in the central nervous system, in association with
a means for gene delivery, to a patient in need thereof to
prevent the accumulation of amyloid-β peptides and the
aggregation of peptides which form amyloid deposits in the
brain.

2.      The method according to claim 1, wherein the
composition is administered by injection intravenously,
intra-arterially, intracranially, or intracephalically.

3.      The method according to claim 1, wherein the
amyloid-β peptide is selected from the group consisting of
amyloid β-peptides having the amino acid sequence of residues
5-44 of SEQ ID NO:1, residues 5-46 of SEQ ID NO:1, residues
5-47 of SEQ ID NO:1, and mixtures thereof.

4.      The method according to claim 1, wherein the
recombinant antibody molecule is end-specific for the N-
terminus of the amyloid-β peptide.

5.      The method according to claim 1, wherein the
recombinant antibody molecule is end-specific for the C-
terminus of the amyloid-β peptide.

. 6.      The method according to claim 1, wherein the
promoter operably-linked to the gene encoding a recombinant
antibody molecule is a βAPP promoter.

7.      The method according to claim 1, wherein the
means for gene delivery in association with the recombinant
DNA molecule comprises a viral vector.

8.      The method according to claim 7, wherein the
viral vector is adeno-associated vector (AAV).

9.      The method according to claim 7, wherein the
means for gene delivery further comprises cationic lipids or
cationic liposomes.

10.    The method according to claim 1, wherein the means for gene delivery in association with the recombinant DNA molecule comprises cationic lipids or cationic liposomes.

11.    The method according to claim 1, wherein the means for gene delivery in association with the recombinant DNA molecule comprises a ligand capable of binding to a cell surface receptor.

12.    The method according to claim 11, wherein the ligand is biotin.

13.    The method according to claim 1, wherein the recombinant antibody molecule is a single chain variable region fragment.

14.    Monoclonal antibody end-specific for the N-terminus of an amyloid $\beta$-peptide.

15.    A recombinant DNA molecule, comprising a gene encoding a recombinant antibody molecule end-specific for the N-terminus or the C-terminus of an amyloid-$\beta$ peptide and a promoter operably linked to said gene, wherein said promoter is capable of expressing said recombinant antibody molecules in brain cells.

16.    The recombinant DNA molecule according to claim 15, wherein said promoter is a $\beta$APP promoter.

17.    A vector comprising the recombinant DNA molecule of claim 15.

18.    A host cell transformed with the vector of claim 17.

19.    A pharmaceutical composition for preventing or inhibiting progression of Alzheimer's Disease, comprising the recombinant DNA molecule of claim 15 in association with a means for gene delivery, and a pharmaceutically acceptable excipient.

20.    The pharmaceutical composition according to claim 19, wherein the means for gene delivery is selected from the group consisting of viral vectors, cationic lipids, cationic liposomes, ligands capable of binding to a cell surface receptor, and combinations thereof.

21.    The pharmaceutical composition according to claim 19, wherein said gene encodes a recombinant antibody

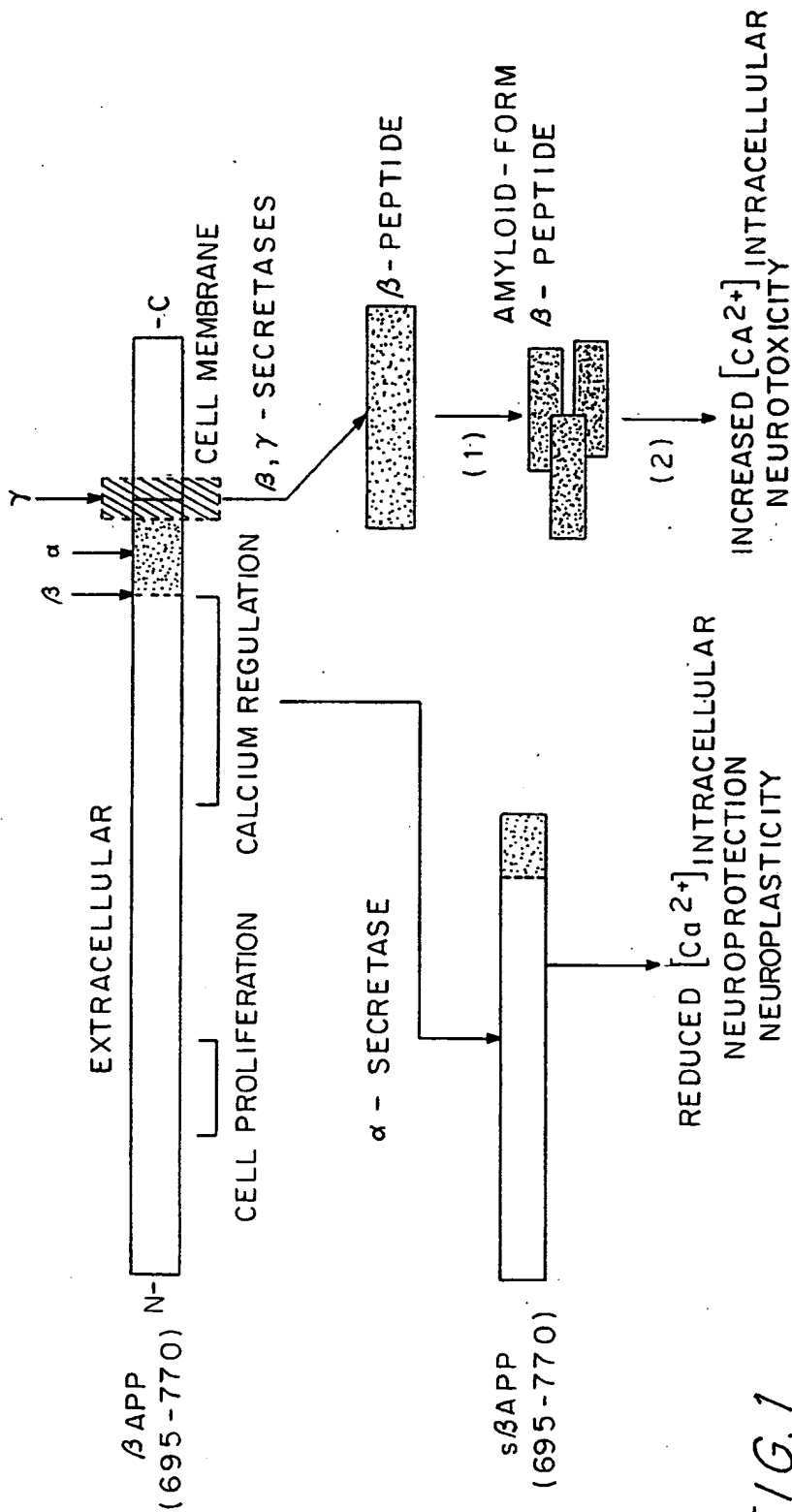molecule end-specific for the N-terminus of an amyloid-β peptide.

22. The pharmaceutical composition according to claim 19, wherein said gene encodes a recombinant antibody molecule end-specific for the C-terminus of an amyloid-β peptide.

βAPP
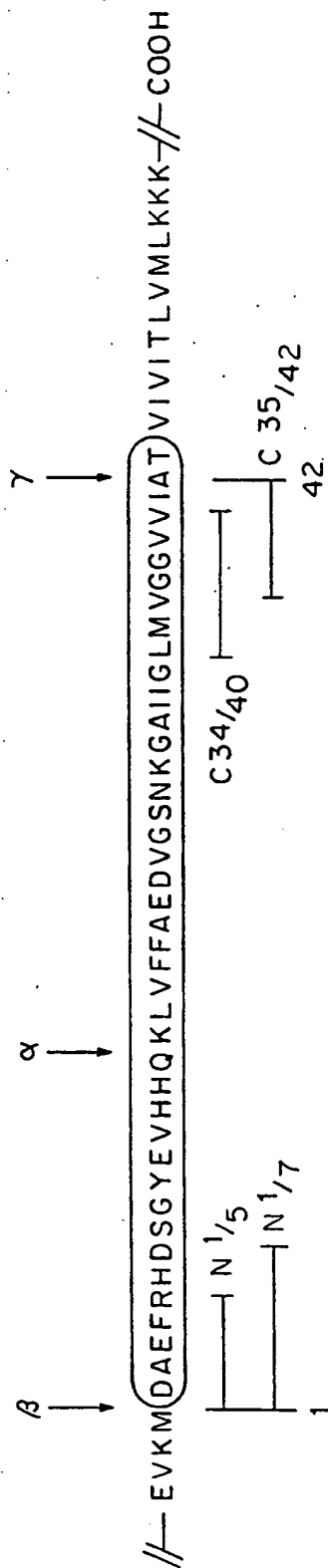(695-770) N-

EXTRACELLULAR

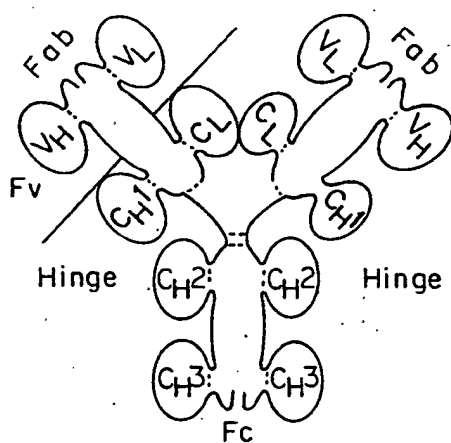CELL PROLIFERATION

CALCIUM REGULATION

CELL MEMBRANE

β α

γ

β,γ - SECRETASES

-C

β - PEPTIDE

(1)

AMYLOID-FORM
β - PEPTIDE

(2)

INCREASED $[Ca^{2+}]_{INTRACELLULAR}$
NEUROTOXICITY

α - SECRETASE

sβAPP
(695-770)

REDUCED $[Ca^{2+}]_{INTRACELLULAR}$
NEUROPROTECTION
NEUROPLASTICITY

FIG. 1

FIG.2

3 / 4

ANTIBODY



FIG. 3A



Fab

FIG. 3B

Fv



FIG. 3C

SINGLE CHAIN Fv (ScFv)



$H_2N - V_H - LINKER - V_L - COOH$

FIG. 3D

4 / 4

## FIG. 4

HEAVY CHAIN cDNA     LIGHT CHAIN cDNA

A                    B          C              D

LP          V$_H$    C$_H$   LP      V$_K$    C$_K$

PCR↓                          ↓PCR

INTERCHAIN LINKER
↓ OVERLAP EXTENSION

A  G                          DH I

LP      V$_H$        ICL        V$_K$    C$_K$

## FIG. 5

190 bp   XbaI                              XbaI

huβAPP                ScFvαAβ              SV40
                                           pA
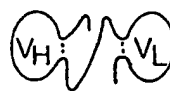
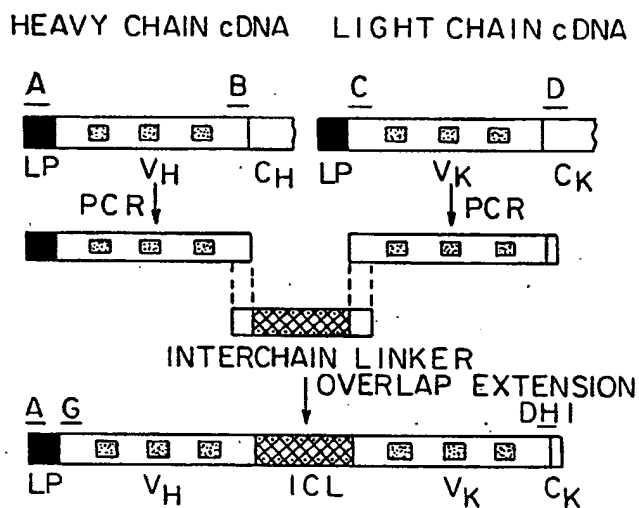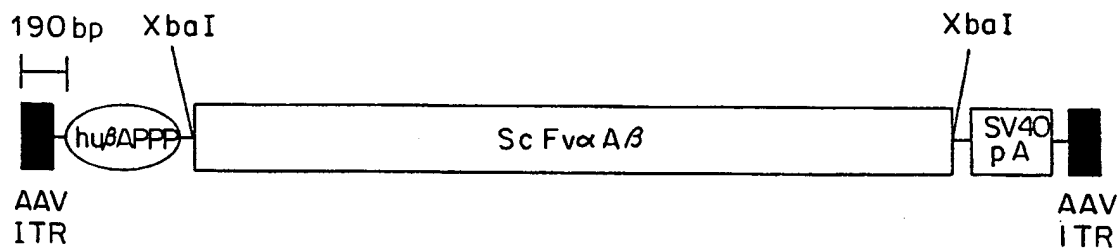AAV                                        AAV
ITR                                        ITR
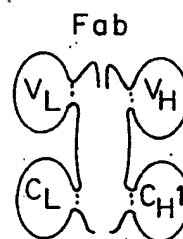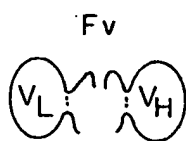
International application No.

PCT/US98/06900

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 48/00; C07H 21/00; C12N 15/00; C12P 21/04, 21/06, 21/08;

US CL :435/69.1, 70.21, 320.1, 326, 331; 514/44; 530/388.1; 536/23.53

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 70.21, 320.1, 326, 331; 514/44; 530/388.1; 536/23.53

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | WO 89/01975 A1 (CELLTECH LIMITED) 09 March 1989, see entire document. | 1-13, 15-22 |
| Y | WO 96/18900 A1 (RAMONT UNIV. AUTHORITY FOR APPLIED RESEARCH AND INDUSTRIAL DEVELOPMENT LTD.) 20 June 1996, see entire document. | 1-13, 15-22 |
| X — Y | EP 0683234 A1 (TAKEDA CHEMICAL INDUSTRIES, LTD.) 22 November 1995, see entire document. | 14 ——— 1-13, 15-22 |
| Y | SOLOMON et al, Monoclonal antibodies inhibit in vitro fibrillar aggregation of the Alzheimer beta-amyloid peptide. Proc. Natl. Acad. Sci. USA. January 1996, Vol. 93, pages 452-455, see entire document. | 1-13, 15-22 |

[X] Further documents are listed in the continuation of Box C.      [ ] See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | | |
| "E" | earlier document published on or after the international filing date | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
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| "O" | document referring to an oral disclosure, use, exhibition or other means | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "P" | document published prior to the international filing date but later than the priority date claimed | "&" | document member of the same patent family |

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 22 JUNE 1998 | 19 AUG 1998 |

| Name and mailing address of the ISA/US | Authorized officer |
|---|---|
| Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 | PATRICIA A. DUFFY |
| Facsimile No. (703) 305-3230 | Telephone No. (703) 308-0196 |

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y,P | SOLOMON et al, Disaggregation of Alzheimer beta-amyloid by site-directed mAb. Proc. Natl. Acad. Sci. USA., April 1997, Vol. 94, pages 4109-4112, see entire document. | 1-13, 15-22 |
| Y | HANAN et al, Inhibitory effect of monoclonal antibodies on Alzheimer's beta-amyloid peptide aggregation. Amyloid: Int. H. Exp. Clin. Invest. 1996, Vol. 3, pages 130-133, see entire document. | 1-13, 15-22 |

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, DIALOG, BIOSYS, EMBASE, DERWENT WPI, JAPIO.
search terms: gene therapy, amyloid, antibody, monoclonal, aggregation, inhibit, prevent, DNA, RNA, host cell, vector, central nervous system, CNS, Alzheimer's, C-terminal, N-terminal.